REMARKS

Claims 1 to 57 are pending, with claims 21 to 57 withdrawn from examination as allegedly drawn to a non-elected invention. The withdrawn claims have been canceled herein. Thus, claims 1 to 20 are pending and presently under examination.

Regarding the rejection under 35 U.S.C. § 112, first paragraph

The objection to the specification and corresponding rejection of claims 1 to 20 under 35 U.S.C. § 112, first paragraph, as allegedly lacking enablement are respectfully traversed. The Office Action sets forth two main grounds for rejecting the claims as allegedly lacking enablement. These two aspects are addressed in turn below.

1. Regarding genetic modification of human embryonic stem (ES) cells

The claims are directed to methods of differentiating progenitor cells such as embryonic stem (ES) cells and hematopoietic progenitor cells and including human progenitor cells such as human stem cells. In rejecting the claims as allegedly lacking enablement, the Office Action asserts that the specification fails to provide an enabling disclosure for the genetic modification of human ES cells.

As support for the alleged lack of enablement, the Office Action cites Eiges et al. (2001), which reports an ExGen 500 transfection protocol as yielding the highest efficiency in transfecting human ES cells. The Office Action notes that the ExGen 500 high efficiency protocol was not disclosed in the subject application. Zwaka et al. (2003) is further cited as emphasizing that there are significant differences between transfection of human and murine stem cells and as evidence that high, stable transfection efficiencies have been difficult to achieve in human ES cells. The Office Action also cites Hanazono et al. (2001) as supporting the position that gene transfer into human hematopoietic stem cells was problematic in 2001, subsequent to the June 2000 priority date of the present invention.

Applicants submit that one skilled in the art would have been able to practice the methods of the invention, including the differentiation of human progenitor cells, without undue experimentation. The specification teaches one skilled in the art how to make and use genetically modified progenitor cells including human progenitor cells. Firstly, the specification teaches how to obtain a variety of mammalian embryonic stem cells including stem cells from mice, cows, primates and humans (page 47, line 5, to page 48, line 28). As set forth in the specification, human embryonic stem cells can be isolated, for example, from human blastocysts; human stem cells also can be obtained, for example, from the cord blood of newborn infants or from adult peripheral blood using well-known procedures (page 48, line 29, to page 51, line 12). Furthermore, methods of introducing a nucleic acid molecule into a progenitor cell such as an embryonic stem cell also were known in the art at the time the invention was made (page 54, line 4, to page 55, line 17). As set forth in the specification, well-known methods for introducing a nucleic acid molecule into a progenitor cell such as an embryonic stem cell include, for example, microinjection, electroporation, lipofection and viral-mediated techniques (page 54, line 4, to page 55, line 17). As corroboration that transfection methods such as electroporation can be useful for transfecting human progenitor cells, Applicants direct the Examiner's attention to the cited reference by Eiges et al., in which the efficiency of transfection into human ES cells was assessed by determining luciferase reporter gene activity (page 515, Figure 1). As shown in Figure 1, luciferase reporter gene activity was observed in the electroporation-transfected H9 human ES cells, indicating that these human progenitor cells were successfully transfected. Thus, the results shown in Eiges et al. corroborate the teachings of the specification by demonstrating that only routine methods such as electroporation would have been required for the skilled person to practice the claimed invention with human progenitor cells.

Regarding "high efficiency" transfection

The Office Action emphasizes that human stem cells have proven more difficult to transfect than murine stem cells and that the specification does not disclose the ExGen 500 protocol described by Eiges et al. as yielding the highest efficiency of several methods compared for transfection of human ES cells.

Applicants respectfully point out that the claimed methods of differentiating progenitor cells do not recite, nor do they require, a particularly "high efficiency" of transfection. Rather, one skilled in the art understands that cell populations stably expressing an introduced nucleic acid molecule can be routinely prepared using, for example, standard methods such as antibiotic selection in order to select for a transfected population of cells. In this regard, the specification discloses, for example, the selection of MEF2C-expressing P19 ES cells using a neomycin resistance gene and selection with Geneticin (see page 68, line 19, to page 69, line 4). Thus, the skilled person understands that, even if progenitor cells were not transfected with particularly high efficiency, one skilled in the art would have been able produce a population of progenitor cells predominantly or uniformly containing a MEF2 polypeptide using only routine methods.

In sum, Applicants maintain that the claimed methods do not recite or require transfection of human progenitor cells with an efficiency that matches the efficiency of transfection of murine progenitor cells, nor do they recite or require "high efficiency" transfection of human progenitor cells. Thus, undue experimentation would not have been required to practice the claimed invention even in the absence of the high efficiency ExGen 500 transfection described in Eiges et al.

2. Regarding in vivo differentiation of embryonic stem (ES) cells

The methods of the invention involve contacting progenitor cells with a differentiating agent in order to differentiate the progenitor cells to produce a cell population containing protected neuronal cells. As stated in the Office Action, the progenitor cells can be differentiated *in vitro* or *in vivo.* While it is acknowledged that ES cells have been successfully directed to differentiate *in vitro,* the Office Action asserts that the state of the art for *in vivo* differentiation of ES cells was undeveloped at the time the invention was made. A 1995 reference by Jacowski is cited in support of alleged art-recognized limitations associated with transplantation of neural tissue.

Applicants submit that undue experimentation would not have been required to practice the claimed methods using *in vitro* differentiation, *in vivo* differentiation, or a combination thereof. In this regard, the specification provides guidance to the skilled person by teaching that cells can be transplanted prior to or during differentiation and that, where cells are transplanted prior to differentiation, the neuronal environment can drive the cells into the desired neuronal cell type due to the presence of appropriate environmental cues (page 59, lines 3-9). Thus, one skilled in the art understands that *in vivo* differentiation does not require identification or isolation of differentiation agents, as is needed for *in* vitro differentiation, but instead relies on differentiation agents naturally present in the native milieu. Furthermore, in contrast to the assertion made in the Office Action, Applicants submit that *in vivo* differentiation of progenitor cells was not an undeveloped art at the time the invention was made. As corroboration that the state of the art for *in vivo* differentiation of ES cells was developed by the priority date of the subject application (June 5, 2000), Applicants provide herewith as Exhibit A Liu et al., "Embryonic Stem Cells Differentiate into Oligodendrocytes and Myelinate in Culture and after Spinal Cord Transplantation," Proc. Natl. Acad. Sci. USA 97:6126-6131 (2000), which demonstrates that ES cells can survive, migrate and differentiate into mature myelin-producing cells in demyelinated adult spinal cord (see paragraph spanning pages 6129 and 6130). As summarized in the abstract, "ES cells were transplanted into the dorsal columns of adult rat spinal cord 3 days after chemical demyelination. In the demyelination site, large numbers of ES cells survived and differentiated primarily into mature oligodendrocytes that were capable of myelinating axons." Similarly, *in vivo* differentiation of progenitor cells is corroborated by work published in early 1998 (Deacon et al., "Blastula-stage Stem Cells Can Differentiate into Dopaminergic and Serotonergic Neurons after Transplantation," Exp. Neurol. 149:28-41 (1998)), attached as Exhibit B. In particular, Deacon et al. demonstrate that totipotent mouse stem cells survived and differentiated into cells with neuronal morphology and expressing neural-specific markers following transplantation into adult mouse or rat brain (page 28, abstract). See, also, page 31, Figure 1, which shows the neurofilaments and neuron-specific enolase (NSE) reactivity observed in intracerebral mouse ES cell grafts in rat striatum. As addition evidence, Applicants provide herewith as Exhibit C work published in December of 1999 (McDonald et al.,

"Transplanted Embryonic Stem Cells Survive, Differentiate and Promote Recovery in Injured Rat Spinal Cord," Nature Med. 5:1410-1412 (1999)). McDonald et al. indicate that embryonic stem cells are one source of undifferentiated cells "that have been derived from several species, including mouse and human, and are capable of differentiation into neurons and astrocytes after being transplanted into the brain" (page 1410, second column, first complete sentence). McDonald et al. further demonstrate in their 1999 publication that multipotent, neural differentiated mouse embryonic stem cells survived and differentiated into astrocytes, oligodendrocytes and neurons following transplantation into rat spinal cord following acute injury (see, for example, page 1410, abstract; and page 1411, paragraph spanning columns). Thus, results presented in multiple publications corroborate that, prior to June of 2000, one skilled in the art would have been able to differentiate progenitor cells such as embryonic stem cells *in vivo*.

Finally, in regard to the cited reference by Jacowski, Applicants submit that this publication does not support the unpredictability of the claimed methods which are practiced by contacting progenitor cells with a differentiating agent *in vivo*. Applicants first note that the cited reference by Jacowski is a 1995 review article which cannot be considered representative of the state of the art in the year 2000, at the time the invention was made. Secondly, Applicants draw the Examiner's attention to the fact that Jacowski states that foetal transplants are exempt from the difficulties that affect *in* vivo differentiation (page 308, section entitled "Foetal transplants seem exempt"). In this section, Jacowski goes on to indicate that human neuroblasts and embryonic neurons have formed axons when transplanted into adult mammalian central nervous systems. Thus, in contrast to the Examiner's assertion, the cited reference by Jacowski demonstrates that the methods of the invention can be practiced by *in vivo* differentiation of embryonic or other progenitor cells.

In sum, given the state of the art at the time the invention was made, the skilled person would have been able to practice the claimed invention without undue experimentation, whether the differentiation was carried out *in vitro, in vivo* or a combination thereof.

Accordingly, it is respectfully requested that the Examiner reconsider and remove the enablement rejection of claims 1 to 20 under 35 U.S.C. § 112, first paragraph.

CONCLUSION

Applicants respectfully request that the Examiner consider the amendments and remarks herein above. The Examiner is invited to call the undersigned agent or Cathryn Campbell if there are any questions.

Respectfully submitted,

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